

ENDOTHELIAL CELL GROWTH FACTOR,
METHOD OF ISOLATION AND EXPRESSION

ABSTRACT OF THE DISCLOSURE

5 A novel growth factor specific for vascular endothelial cells has been identified in conditioned medium of bovine pituitary derived folliculo stellate cells. This factor, named folliculo stellate derived growth facto (FSdGF) or vascular endothelial growth factor (VEGF), was purified to homogeneity by a combination of heparin sepharose affinity chromatography, Bio Gel P-60
10 exclusion chromatography, Mono S ion exchange chromatography and hydrophobic chromatography on a C4 reverse phase HPLC column. The factor is also found in the murine AtT-20 cell line.

15 Alternatively, the growth factor is purified by a first reverse phase HPLC using acetonitrile gradient followed by a second reverse phase HPLC using an isopropanol gradient. FSdGF, having a molecular weight of about 43,000 da, was characterized as a glycoprotein composed of two homologous sub units with MW of about 23 kDa. FSdGF was a potent mitogen for vascular endothelial
20 cells with activity detectable at 10 pg/ml and saturation at 500 pg/ml. It did not stimulate the proliferation of other cell types such as bovine corneal endothelial cells, adrenal cortex cells, granulosa cells, BALB/MK cells or BHK-21 cells. Microsequencing revealed an amino terminal
25 sequence containing no significant homology to any known protein. The release of FSdGF by pituitary cells and its

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unique target cell specificity indicate that FSdGF is
useful in angiogenesis.

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